

Supplementary Table 1: Patient characteristics

		<u>Cohort (n = 95)</u>
Age at initial diagnosis	Median (range)	62 (26-83)
Sex	Female	57 (58.8%)
	Male	38 (39.2%)
Smoking status	Current	46 (48.4%)
	Former	31 (32.6%)
	Never	13(13.7%)
	Unknown	5 (5.3%)
Pack-years	Median (range)	40 (2.5-160)
Stage at initial diagnosis (AJCC 7th edition)	I-III	49 (50.5%)
	IV	44 (46.3%)
Histological subtype	Adenocarcinoma	60 (63.2%)
	Squamous	11 (11.6%)
	Small cell	10 (10.5%)
	Other NSCLC	14 (14.7%)
Molecular alterations	KRAS	9 (9.5%)
	EGFR	6 (6.3%)
	ALK	3 (3.2%)
	None/NA	76 (89%)

Supplementary Table 2: Tissue characteristics

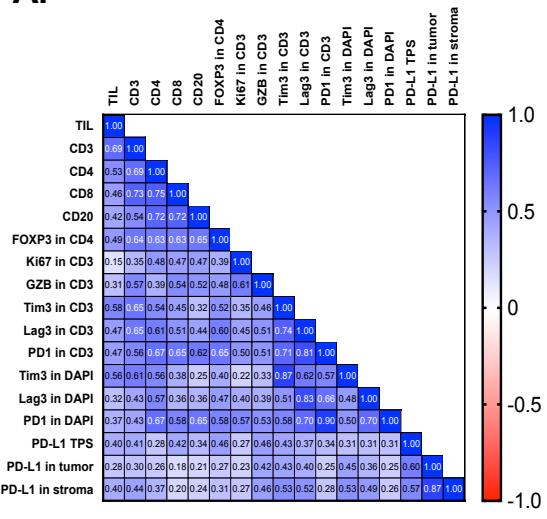
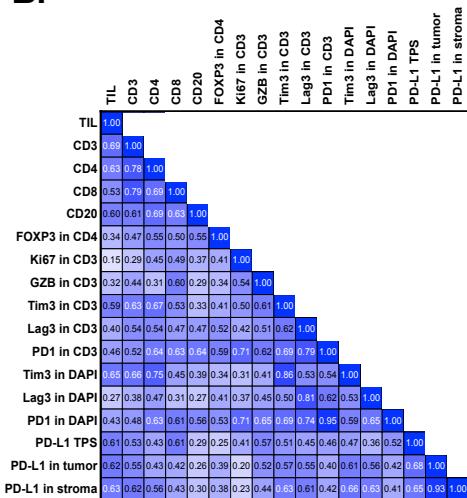
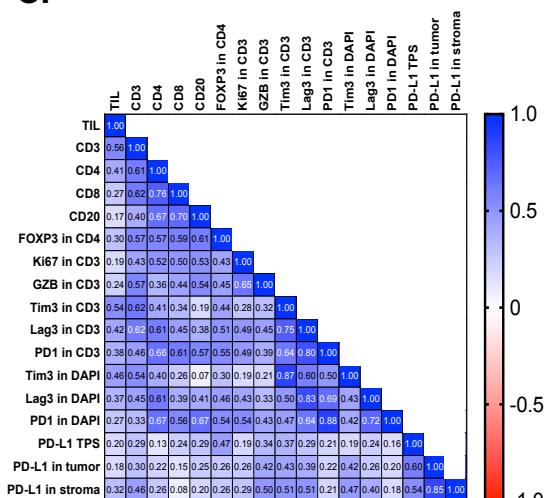
		Total samples n = 106
Tissue characteristics	Primary lung tumors	41 (38.7%)
	Brain metastases	65 (61.3%)
	Paired lung-brain metastasis	11 (10.4%)
Temporal relationship of paired samples	Synchronous	3 (27.3%)
	Metachronous	8 (72.7%)
Treatment prior to tissue sampling	Treatment prior to tissue sampling	29 (27.4%)
	Prior radiation at site	20 (18.9%)
	Chemotherapy within 30 days	17 (16.0%)
	Prior immunotherapy	2 (1.9%)

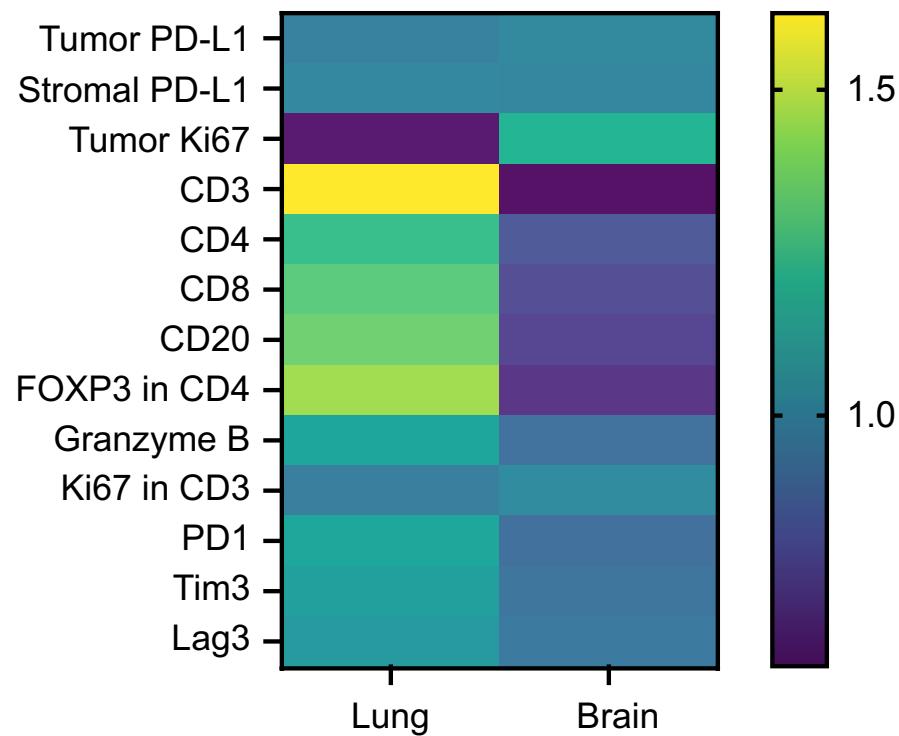
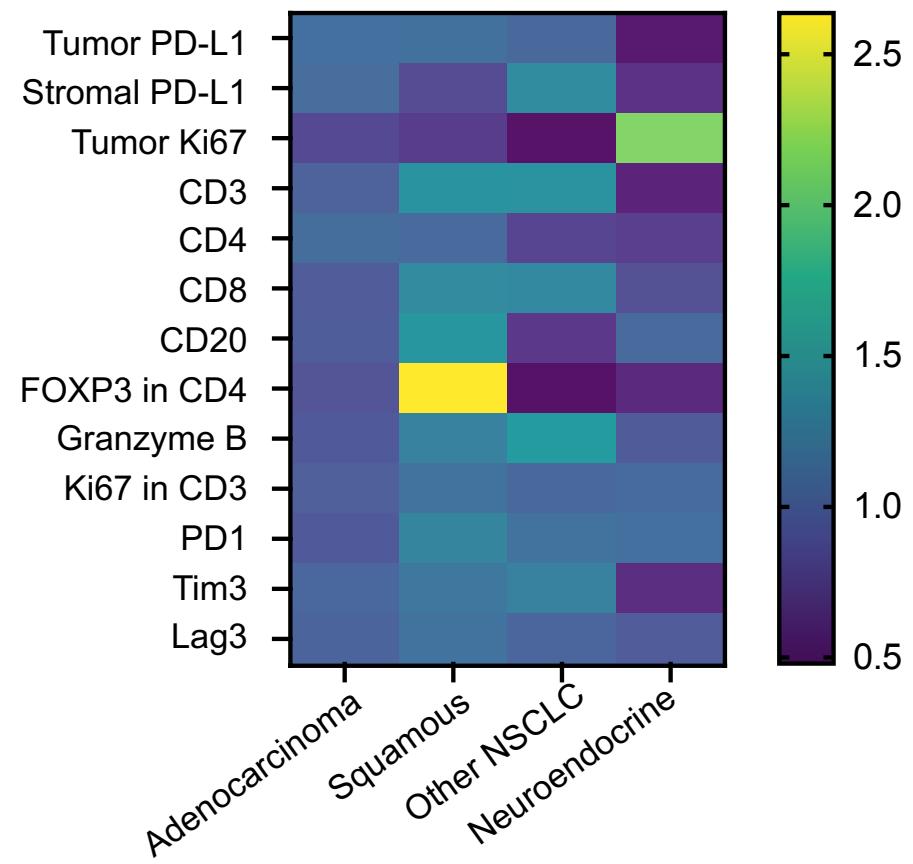
Supplementary Table 3: Clinicopathologic associations with immune markers in brain metastases

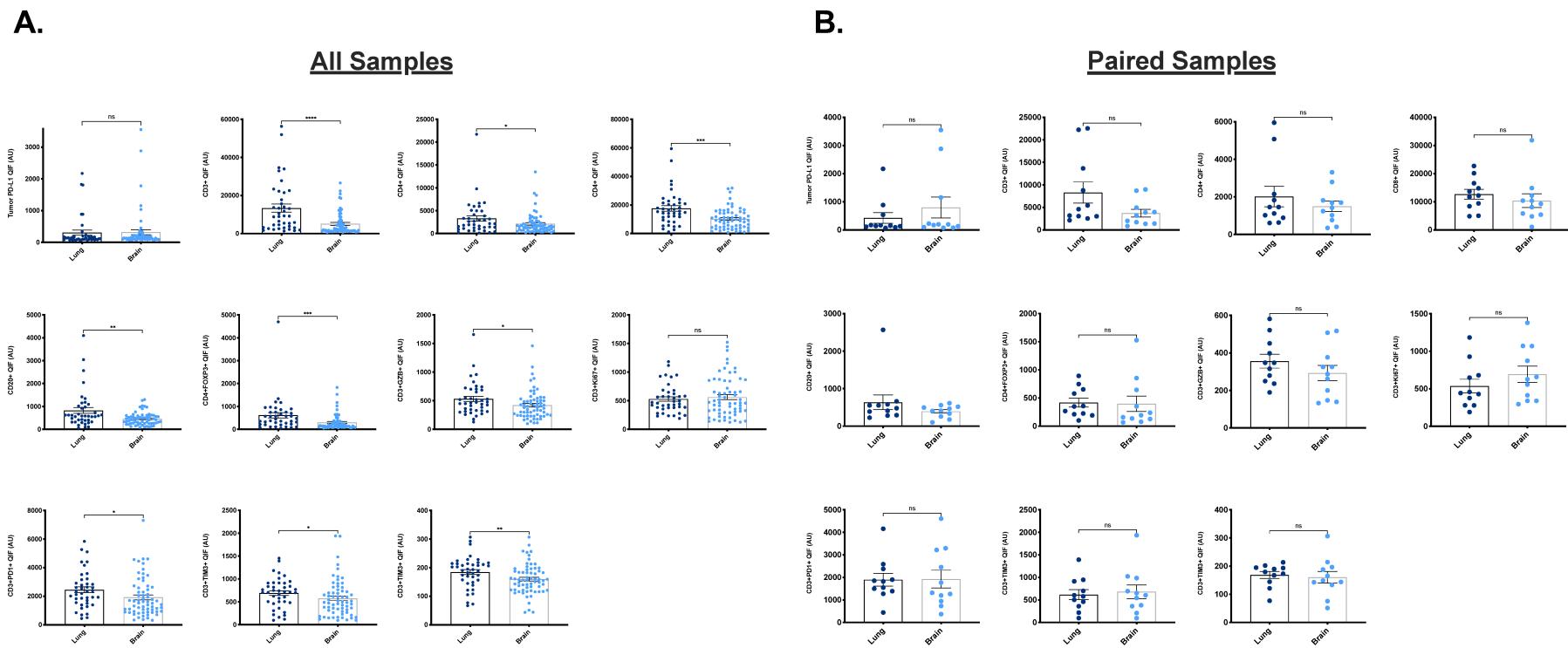
BRAIN METASTASES	PD-L1	CD3	CD4	CD8	CD20	FOXP3	GZB in CD3	Ki67 in CD3	Tumor Ki67	PD1	TIM3	LAG3	
Smoking status	<i>n</i>												
Never/Unknown	11	113 <i>P</i>	2864 <i>P</i>	1650 <i>P</i>	12342 <i>P</i>	365.6 <i>P</i>	187.1 <i>P</i>	230.4 <i>P</i>	323.8 <i>P</i>	447.6 <i>P</i>	1097 <i>P</i>	607.1 <i>P</i>	
Current/Former	54	149.3 0.136	2280 0.369	1462 0.934	9243 0.51	460.3 0.302	172.3 0.369	409.2 0.081	471.9 0.266	760.1 0.069	1562 0.286	486.5 0.484	157.5 0.623
Stage													
I-III	24	170.5 <i>P</i>	1949 <i>P</i>	1320 <i>P</i>	8593 <i>P</i>	444.5 <i>P</i>	189.6 <i>P</i>	340 <i>P</i>	420.1 <i>P</i>	726.2 <i>P</i>	1289 <i>P</i>	467.9 <i>P</i>	153.6 <i>P</i>
IV	41	132.3 0.048	2563 0.165	1639 0.419	9829 0.861	420.9 0.618	168.8 0.842	405 0.539	507.9 0.359	766.3 0.558	1603 0.539	497 0.327	156.8 0.453
Subtype*													
Adenocarcinoma	44	389.4 <i>P</i>	4771 <i>P</i>	2194 <i>P</i>	9901 <i>P</i>	406.7 <i>P</i>	291 <i>P</i>	387.2 <i>P</i>	537.1 <i>P</i>	888.2 <i>P</i>	1754 <i>P</i>	599 <i>P</i>	159.3 <i>P</i>
Squamous	3	155.9 0.545	12904 0.086	3435 0.755	12093 0.456	482.8 0.26	789 0.297	528 0.511	504.7 0.828	501.4 0.009	1954 0.515	824.6 0.033	179 0.776
Other NSCLC	6	116.7	6432	2011	13964	473.7	250	585.1	602.4	647.1	2275	723.4	160.2
Neuroendocrine	12	175.7	3526	1525	9395	654.7	231.6	435.8	646.5	2714	2329	349.4	155.9
Prior treatments													
None	39	130.9 <i>P</i>	3518 <i>P</i>	1685 <i>P</i>	10917 <i>P</i>	411.3 <i>P</i>	188.4 <i>P</i>	373.3 <i>P</i>	457.6 <i>P</i>	759.1 <i>P</i>	1716 <i>P</i>	651 <i>P</i>	162.7 <i>P</i>
Any	26	182.5 0.084	1691 0.022	1021 0.051	8466 0.288	463.4 0.386	175.3 0.493	392.2 0.649	522.8 0.897	710.4 0.984	1177 0.051	363.3 0.003	144.7 0.031
Prior XRT													
None	46	130.4 <i>P</i>	2864 <i>P</i>	1571 <i>P</i>	9250 <i>P</i>	407.8 <i>P</i>	172.3 <i>P</i>	379.8 <i>P</i>	471.9 <i>P</i>	773.5 <i>P</i>	1678 <i>P</i>	573.2 <i>P</i>	158.7 <i>P</i>
Yes	19	190.1 0.023	1698 0.143	1022 0.248	9123 0.899	484.8 0.23	187.1 0.389	400.8 0.631	458.7 0.705	639.7 0.531	1097 0.079	428.8 0.044	141.6 0.099

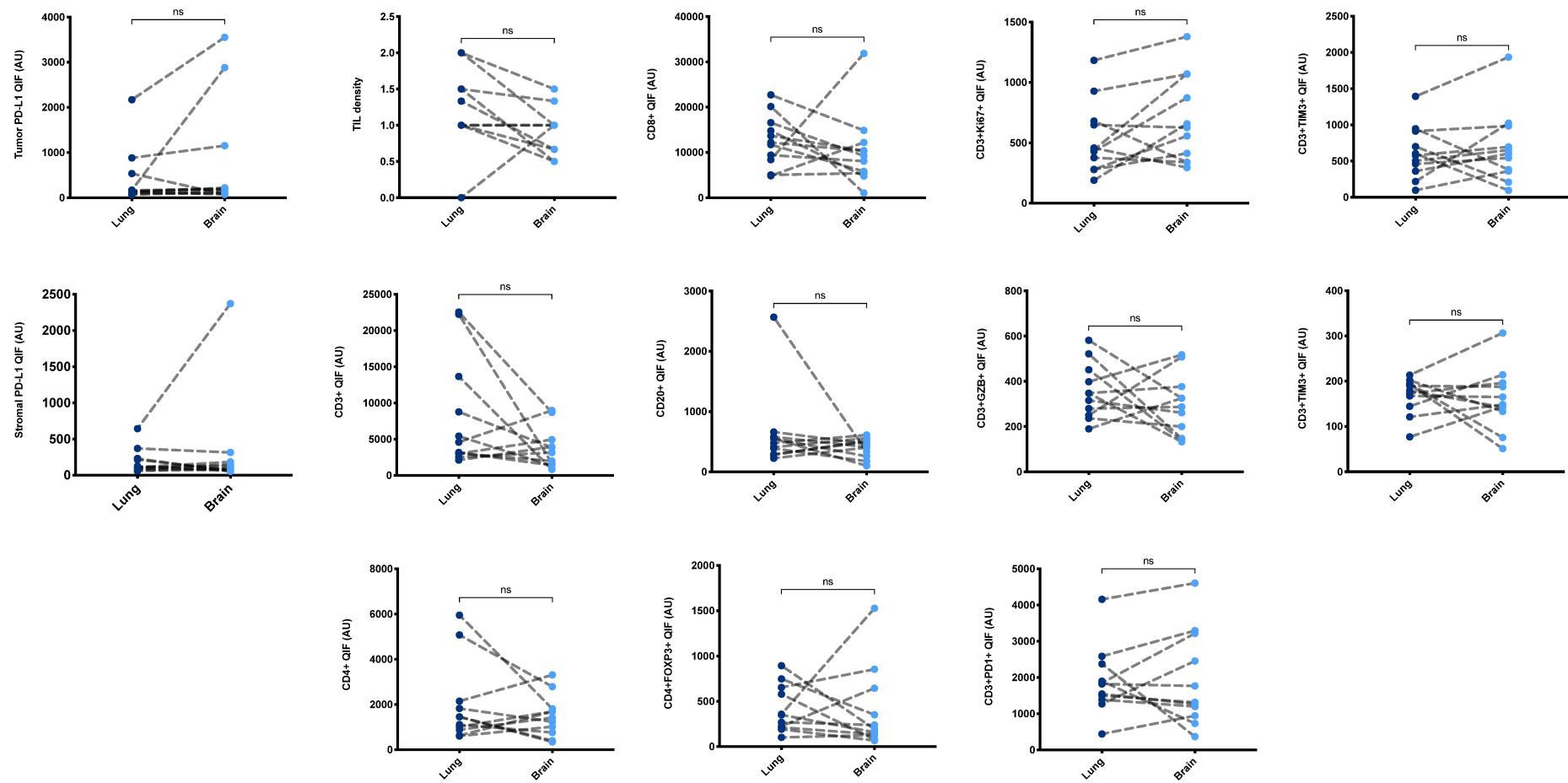
* Mean displayed

Supplementary Figure 1: Spearman correlation coefficients between immune markers

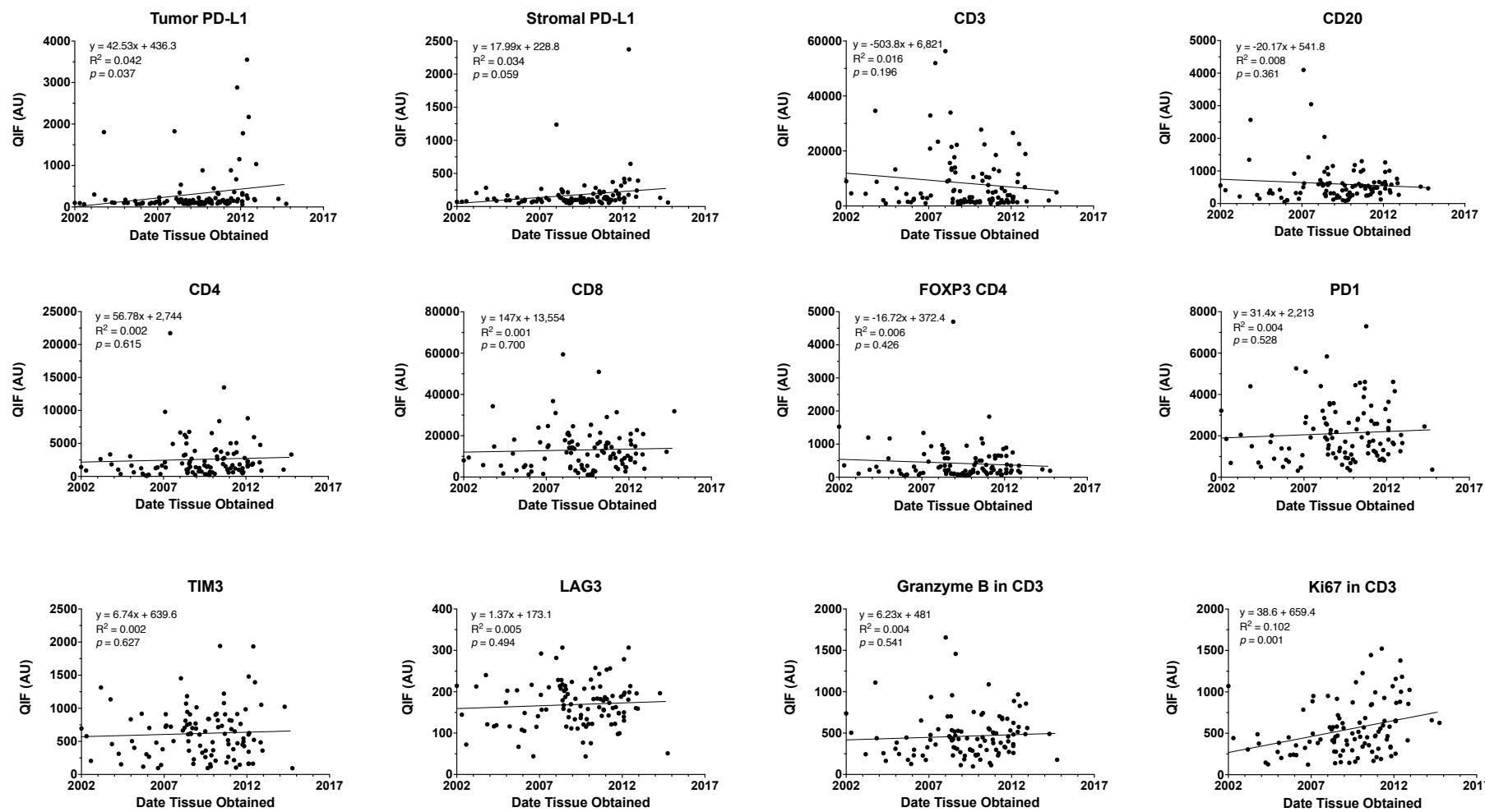
A.**All tissues****B.****Lung****C.****Brain**

Supplementary Figure 2: Heatmap of immune marker expression**A.****B.**

Supplementary Figure 3: Scatter plots of immune marker expression as measured by quantitative immunofluorescence

Supplementary Figure 4: Immune marker expression between paired primary lung and brain metastases

Supplementary Figure 5: Marker expression by date of tissue acquisition



Supplementary Figure 6: Survival curves in primary lung tumors